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In two series, the effects on performance were tested 30 min after the i.m. injection of pyridostigmine (0.2-0.8 mg/kg) and 20 min after i.m. physostigmine (0.02-0.08 mg/kg) in experimentally naive marmosets. The behavioral tasks in series 1 were hand-eye coordination, reaction time and a discrete-trial two-choice visual discrimination task. For series 2, the reaction time task was dropped because detection was unreliable and a substitute was introduced into the discrimination task, which was transformed into an operant task. All tasks were trained and tested in succession in one session per day. This appeared difficult for the animals and only approximately 50% of the animals reached an acceptable performance level at which testing became meaningful. On hand-eye coordination, the lowest doses of pyridostigmine (0.2 mg/kg) and of physostigmine (0.02 mg/kg) were still above the no-effect level. This was also true for the effect of physostigmine

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(0.02-0.04 mg/kg) in the operant discrimination task, but in this task pyridostigmine (0.2-0.4 mg/kg) had no effect, suggesting that the discrimination task is predominantly CNS-mediated. These dose-levels are at least 4 times lower than those found by British scientists (personal communication E.A.M. Scott and G.D. d'Mello). In a 2-day joint critical assessment session with these scientists, in our laboratory, suggestions aimed at increasing the number of animals that reach an acceptable level of performance, at which testing becomes meaningful, were incorporated in the training procedures for a subsequent series. (KR) ←

SUMMARY

In two series, experimentally naive marmosets (Callithrix jacchus) were trained and tested by a robot to exclude human interference as much as possible. In these experiments the, effects of pyridostigmine (0.2-0.8 mg/kg, i.m.) and of physostigmine (0.02-0.08 mg/kg, i.m.) on performance of several tasks were investigated. In series 1, the effects on performance were tested with an acquired hand-eye coordination task, a reaction time task, and a discrete-trial two-choice visual discrimination task. In series 2, the reaction time task was dropped, and a substitute ("motor speed") was incorporated in the discrimination task. The latter task was transformed into an operant task. In both series, all tasks were trained in succession in one session per day. These tasks, especially those in series 2, appeared difficult for the animals to learn, and after 2-3 months of training, only approximately half of the animals reached an acceptable level of performance at which the influence of carbamates could be tested in a meaningful manner. Consequently, the number of animals tested is small.

After it had been established that repeated i.m. injections with saline caused no performance decrements performance tests of the animals started 20 min after physostigmine (dose range 0.02 - 0.08 mg/kg, i.m.) and 30 min after pyridostigmine (dose range 0.2 - 0.8 mg/kg, i.m.).
The lowest doses of both carbamates tested were above their no-effect levels; 0.02 mg/kg physostigmine, as well as 0.2 mg/kg pyridostigmine, affected hand-eye coordination, and 0.02-0.04 mg/kg physostigmine (but not 0.2-0.4 mg/kg pyridostigmine) caused a large deficit in operant discrimination performance in series 2. The lowest doses of both carbamates were 4 times lower than those that caused a significant effect on a visually guided reaching task as found by British scientists (personal communication, E.A.M. Scott and G.D. d'Mello). This higher sensitivity to the disruptive effects of carbamates on performance might be explained by the greater difficulty of the tasks in the present experiments. Based on the results for the few animals tested, the dose-ratio of physostigmine/pyridostigmine for the discrimination task seems to be much larger than that for the hand-eye coordination task. This may suggest that the discrimination task makes a larger demand on CNS functions, which are less easily disrupted by low doses of pyridostigmine because this compound is thought to hardly penetrate into the brain due to its quaternary nitrogen. In view of the disappointing number of animals that ultimately reached an acceptable level of performance, the British colleagues mentioned above, who have long experience with marmoset behavior, were invited to our laboratory. After a 2-day critical analysis of the procedures and the data obtained so far, several suggestions are incorporated in a next series, which will most likely accelerate training and increase the number of animals that reach a stable, high level of performance. There is a clear need for more test results before definite conclusions can be drawn.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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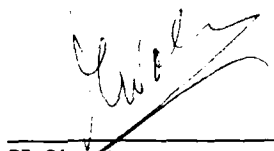

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I. INTRODUCTION

In an earlier study with rats (Wolthuis and Vanwersch, 1984), it was found that at dose-levels of 30% of the LD_{50}, neither soman, sarin, tetraethyl pyrophosphate (TEPP), physostigmine nor pyridostigmine had an effect on running speed or on a number of step parameters when the animal walked in a hollow rotating wheel. In contrast, at lower dose-levels, soman, physostigmine, and, to a lesser extent, pyridostigmine, dose-dependently disrupted performance of a recently acquired shuttlebox task and a motor coordination task, and also disrupted open field behavior. Sarin and TEPP were ineffective, even at doses of 30% of the LD_{50}. The dose-levels at which these inhibitors cause overt symptoms are much higher. It was concluded that after exposure to low doses of cholinesterase inhibitors, different types of behavior, particularly those involving higher CNS structures, may be disrupted at dose-levels that do not cause physical incapacitation.

The absence of detectable effects with a dose of 30% of the LD_{50} of sarin and TEPP did not come as a surprise, since these agents have predominantly- -but not exclusively- -peripheral effects (Meeter and Wolthuis, 1968; Wolthuis et al.,1981b). It was expected that soman, which acts predominantly on the CNS, as well as physostigmine, which easily passes the blood-brain barrier, causes behaviorally disruptive effects at low dose-levels. However, it was surprising that pyridostigmine also caused behavioral decrements at dose-levels below 30% of its LD_{50} value, albeit at a dose that was 2.5-3 times higher than that of physostigmine on a molar basis. This suggests that more pyridostigmine reaches the brain than was hitherto assumed, which in turn may explain why prophylaxis with a carbamate that allegedly acts peripherally protects against the lethal - -although not against the incapacitating- -effects of a predominantly centrally acting inhibitor like soman.

To increase the likelihood that these findings can be extrapolated to man, it is imperative that they are substantiated by similar findings in at least one other species, preferably one that is closer to man. For this, the marmoset was chosen for a number of reasons. First, the marmoset is considered ideal for behavioral laboratory studies (Grist, 1974; Stevenson, 1977). It has become quite popular in recent years (see e.g., Baker et al., 1983; Annett et al., 1983; Kendrick and Dixson, 1984; Abbott, 1984; Engel, 1985), and its behavior is sensitive to cholinergic manipulation (Baker et al., 1984; Ridley et al., 1984a, 1984b; Ridley et al., 1985; d'Mello and Duffy, 1985). Directly relevant for the present investigation are the results of the latter authors, who found that 35-55% of the LD_{50} of sarin disrupted a visually guided reaching task, an effect that could not be explained by indirect effects on motivation or gross mobility. In essence, their elegant and simple technique formed the basis for the hand-eye coordination task used in the present experiments. Second, marmosets are sensitive to carbamate prophylaxis (Dirnhuber et al., 1979), similar to rhesus and in contrast to rodents. Third, in vivo and in vitro studies showed that the marmoset responded to oxime therapy differently from mice, rats, guinea pigs and dogs, but in studies in vitro their neuromuscular preparations responded in a similar way to those of rhesus and man (Wolthuis et al., 1981a; Smith and Wolthuis, 1983; Van Helden et al., 1983). In addition, other reasons, such as the low levels of scavenging blood carboxylesterases (similar to man, different from rodents), the reproduction rate of marmosets in captivity, as well as the size

and manageability of the animal, led to the choice of the marmoset as the experimental animal for the present study. On the basis of the earlier investigations in rats mentioned above (Wolthuis and Vanwersch, 1984), it was expected 1) that performance of tasks involving higher CNS functions would be disrupted at lower dose-levels of cholinesterase (CHE) inhibitors than simpler reflex-like motor tasks involving fewer synapses; 2) that soman and physostigmine, due to their actions on the CNS, would cause such a disruption at a lower dose (% of LD_{50}) than would sarin or pyridostigmine, respectively, which predominantly- -but not exclusively- -act peripherally; 3) that, due to tolerance phenomena, the effects detectable 30 min after injection of the organophosphate CHE inhibitors would have disappeared 24 h later; and 4) that at those dose-levels that begin to disrupt behavior, the blood CHE will be only moderately inhibited, and neuromuscular transmission will be hardly affected.

The objective of this study is to contribute to an assessment of the risk that exposure to CHE inhibitors may cause subtle disruptions of higher CNS functions that go unnoticed because physical signs are absent. Particularly suspect are those compounds that act on the CNS at low dose-levels. Such subtle disruptions of CNS functions in man might affect decison-making, logic, memory, and other processes, which are all vital for complex operations.

II. MATERIALS AND METHODS

II-1 ANIMALS (Callithrix jacchus)

Preceding this study, to gain some experience with the possibilities and limitations of marmoset behavior, we obtained 10 male animals of various ages from the Primate Center TNO (PC-TNO). In the course of 1 1/2 years, these animals were subjected to i.m. injections with sterile saline and were exposed to several test conditions. The general conclusion was that the animal could be trained on quite a number of tasks (as was expected on the basis of the literature), albeit that it is high-strung and easily distracted. Hence, we chose to use a robot-driven task to eliminate human influence as much as possible. Apart from vitamin injections and the drawing of blood samples under ketamine anaesthesia for blood tests during a quarantine period, which took place before testing began, all marmosets were experimentally naive and had not been treated with other drugs.
Due to its rapidly increasing popularity as an experimental animal, it unexpectedly appeared almost impossible to obtain sufficient quantities of marmosets of the desired age and sex.

Series 1 was started with 8 male animals, which had been reared at the PC-TNO. Two were rejected soon; one because it did not like the marshmallow-like reward, the other because it appeared extremely right-handed and oriented to the right. The latter animal consistently made scores of 100% at the right window (see below) and zero scores at the left window; attempts to change this failed. Of the six animals that were left, two failed to reach an acceptable level of performance in 60 sessions. Consequently, we were left with four animals. Their ages were 36, 23, 17 and 25 months, and they weighed 387, 292, 506 and 488 g, respectively.

In series 2, the test procedures were partly adapted because of the meager results in series 1 (see below). This series was started with 12 animals (8 males and 4 females), all obtained from the PC-TNO. In 60 sessions only five animals reached an acceptable performance: two sisters of 61 months weighing 380 and 374 g, two brothers of 19 months weighing 449 and 434 g and one female marmoset of 25 months weighing 377 g.

For series 3 we were finally successful in obtaining 31 male marmosets from Charles River Wiga GmbH, Sulzfeld, W. Germany. Unfortunately, since these animals were not obtained from TNO, regulations required that these animals undergo a 3-month period of quarantine, when repeated blood tests, faeces tests, vitamin injections, Mantoux reactions and general health inspections were carried out. A 3-month interruption (June, July, August 1989) of the program was unavoidable, since behavioral training of these high-strung animals was impossible during a period in which all these veterinary manipulations took place. Training started on September 1, 1989.

II-2 APPARATUS (see figure 0)

In essence, the apparatus consists of a programmable robot, centrally situated on a platform and placed symmetrically between two identical test-panels. The square elevated platform runs on rails in such a way that the test-panels are guided, in a cage by cage manner, along a row of cages situated on both sides of the rails. Each cage contains one marmoset. The robot and test-panel are connected on-line with a hard-disk containing AT-PC computer. Attached to

the platform are two television cameras, one horizontal and one vertical, that are connected with two monitors in another room. Thus, the animals can be (and generally are) constantly watched while performing.

The robot holds an 8.5 cm stainless steel suction tube (diam. 4 mm) that contains an optic fiber. For each trial, the robot moves to a plateau with 120 little wells, each containing a little (diam. approx. 5 mm) marshmallow-like reward, sucks one reward onto the end of the tube and moves the reward into the starting position behind the test-panel. The optic fiber guides infrared light, which reflects against the reward sucked onto the tube. The reflected light is used to detect the presence or absence of the reward at the end of the tube and is instrumental in registering its time of removal.

Each test-panel that slides in front of the steel rods of the cage (see below) at the beginning of each test session contains a small loudspeaker, right and left alpha-numerical displays. Below each display is a window that can be opened and closed by a pneumatically driven photocell-guarded vertically sliding door. In this door a detection device is constructed which, on the basis of reflection, detects the presence of the animal in front of the door. Although tested thoroughly at the beginning of the experiment, it appeared later that this gadget did not operate reliably; it was too dependent on the posture of the animal.

For series 2, small holes were made in the right and left sides of the test-panel through which motor-driven handles could be introduced into the cage. Attached to the end of the handle that protrudes into the cage is a 1-2 cm chain. The purpose of the chain is to make it more interesting for the animal to play with the handle and to tempt the animal to pull it, hereby activating microswitches that lead to opening of the window. The reward, presented at the other side of the window, then becomes visible and can be grasped by the animal.

On the inner side of the test-panel, a photocell-guarded trough is constructed. This allows for the detection of the falling reward if the animal does not properly grasp the reward and allows it to drop or if the reward is jettisoned from the suction tube.

The cages (l x w x h = 24 x 24 x 32.5 cm) are made of wiremesh, except for the side facing the panel, which consists of horizontal stainless steel rods, spaced wide enough apart so that the animal can stick its arm at full length out of the cage; i.e., it can easily grasp the reward through the open windows of the test-panel when presented. Perpendicular to and at the middle of the rods a vertical wiremesh partitioning screen (from top to bottom) is present that projects 7 cm into the cage. This screen prevents the animal from sitting facing the middle of the test-panel and forces the animal to make a clear choice between the right or left window.

II-3 TRAINING AND TESTING

SERIES 1

In this series three tests were used: 1) performance on a discrete-trial two-choice visual discrimination task, 2) performance on a hand-eye coordination task and 3) performance on a simple reaction time task. During each training and test session, these tests were applied sequentially; i.e., first all animals were trained on hand-eye coordination, then on reaction time and

subsequently on the discrimination task. (For practical reasons the discrimination task is described first.)

The discrete-trial two-choice visual discrimination task.

Five days per week the animals were subjected to one session per day, consisting of 20 trials in succession. A session started when the test-panel was in place. At the beginning of each trial, a non-directional sound signal (piezo-generated, sinusoidal, 3 KHz) was presented, intended to alert the animal. Immediately thereafter, the left or right (quasi-randomly determined) alpha-numerical display was switched on and both windows were slid open. When the animal had not retrieved the reward within 1140 msec (± 2 msec), the reward was jettisoned from the suction tube, the window closed and a new trial began. NB.: the unusual time period of 1140 msec was due to a time setting of 1000 msec plus the lag time of 138-142 msec it took to change (electric valve operated) from suction to sufficient air pressure to jettison the reward from the tube.

In series 1, the following parameters were automatically registered: N = the number of trials; P = the animal is present in front of the correct window; AT = attempts, i.e., the animal sticks its arm through the window; F = the number of failures, i.e., the animal removes the reward from the suction tube but drops it (into the trough); H = the number of hits, i.e., the animal successfully retrieves the reward from the suction tube and eats it in almost 100% of the cases, as has been checked repeatedly via the TV monitors. The percentage of hits, expressed as $H/N \times 100\%$, is taken as the score and used as a criterion to judge the performance of the animal.

The hand-eye coordination test

This test started after the test-panel was in position; there was one session per day, each consisting of 10 trials in succession. Only one window, e.g., the right window, was used, per daily session. The next day testing occurred only at the left window, the day thereafter at the right window again, etc. This was done to accustom the animals to both windows. It also facilitated the detection of extreme and incorrigible right- or left-handedness (see above). Each trial started with a sound signal; immediately thereafter the alpha-numerical display over the window used that day switched on, and the window opened. At that time, the suction tube with the reward attached was in a ready position, 7.5 cm to the right of the window and out of sight of the animal, and started to move from the right to the left at a speed of 25 cm/sec. The trajectory of the reward followed approximately a horizontal line through the middle of the opened window. A hit was registered when the animal successfully retrieved the moving target from the suction tube. As in the discrimination test, the attempts and failures were also registered. The score was calculated as the percentage of hits out of the maximum of 10 possible:

$$H/N \times 100\%$$

The reaction time test

The intention of this test was to try to induce the animal to grasp the reward as fast as possible, analogous to the simple reaction time test successfully employed for testing humans. Each day one session was held, consisting of 10 trials; the right or left window was used, as in the hand-eye coordination test. The sequence at the beginning of each trial was essentially the same as

that mentioned above: a sound signal was presented and at the same instant the alpha-numerical display switched on. It was intended to open the window when the presence of the animal in front of the window had been detected, and then, by shortening the time period that the window was open, to induce the animal to retrieve the reward as fast as possible. Although the method initially seemed to produce the desired results, upon closer analysis it appeared that 1) the detection of the animal in front of the window was unreliable, since it depended too much on the posture of the animal, 2) the results were quite variable, and 3) for ill-understood reasons the animals gave up when it was attempted to shorten the time of access to the reward ("window open" period) below values of 1140 msec, whereas it appeared that during discrimination testing they were repeatedly successful in grasping the reward in half that time. Hence, this test was dropped as a separate test in series 2; a substitute for the reaction time test was incorporated in the discrimination test (see below). However, for the sake of completeness, the results of this reaction time task are reported (see results). They are expressed as the number of times (out of 10 possible) that the animal was successful in retrieving the reward within 2.00 sec.

SERIES 2

Since the training in series 1 (see section II-1) led only to the desired performance level in 4 out of 6 animals (2 had been rejected earlier for reasons mentioned above), it was decided to leave the hand-eye coordination task essentially intact, but to change the procedure of the discrimination task. This was not only done to incorporate a substitute (motor speed) for the reaction time task, but also to have a more precise estimate of the time it took the animal to make a discriminatory choice (choice time).
To achieve this, two small holes were made in the test-panels, lateral to the tops of the alpha-numerical displays. Through each hole a motor-driven handle could be presented into the cage. Each handle was provided with two microswitches, one that detected when the full length of the handle projected into the cage, and a second that detected when the marmoset pulled the handle. In essence, the task was thereby transformed into an operant task.

The (operant) discrete-trial two-choice visual discrimination task

Immediately after the sound signal, one alpha-numerical display switched on and two handles were simultaneously introduced, one into the upper right and another into the upper left part of the cage. When the animal pulled the correct handle, i.e., the handle on the side at which the alpha-numerical display was on, both windows slid open and then the animal could grasp the reward presented, which was visible through the window in front of it. If the animal pulled the handle at the wrong side, i.e., where the alpha-numerical display was not illuminated, the windows remained closed, the handles were retracted and the lit display was switched off. Immediately thereafter the display (on the same side as before) switched on again and the handles were again introduced; i.e., the animal could correct its mistake.

In addition to the parameters N, AT, F and H mentioned before, there were now a number of additional parameters:
Pg = the number of times the animal first pulled the correct handle;
Pa = the number of times the animal first pulled the incorrect handle or persevered in pulling the incorrect handle before pulling the correct one;
Tl = choice time, i.e., the time that elapsed between the moment that the handle was completely introduced into the cage and the moment when the animal pulled the handle;

T2 = reaction time or rather motor speed; i.e., the time that elapsed between the moment that the windows were completely open (photocell-detected) and the moment that the reward was removed from the suction tube.
Apart from the use of AT, T1 and T2, the main criterion and score for judging the performance of the animals was:

$$H/(Pg + Pa) \times N/20 .$$

This is a hard criterion, since it does not take into account the number of failures (F, registered separately): i.e., an animal may choose and pull the correct handle, it may make an attempt and it may be successful in grasping the reward and removing it from the suction tube, but if the animal, after all these correct reactions drops the reward several times, its score will be low.

II-4 TEST SCHEDULES

In both series 1 and 2, once the performance of the animals had reached an acceptable level, the animals were injected i.m. with sterile saline. Thirty minutes later the performance of the animals was tested. This was repeated several (usually 2-3) times, i.e., until the animals upon testing showed no or negligible disruption of their performance. For series 1, this point was reached at session 65*, for series 2 at session 91*. The large number of sessions needed in series 2 was due to a number of technical mishaps during training: after these were dealt with it took some time before the animals picked up the routine again.

SERIES 1

The tests in session 66 (session 1 in the figures) were control tests; a saline injection was given and performance was tested, followed 3 days later by performance tests 30 min after the i.m. injection of pyridostigmine or 20 min after i.m. injection of physostigmine. Hand-eye coordination data of one animal were erratic before testing started; these data were omitted from the results. The doses and time intervals to testing used were based on information on blood CHE inhibition patterns (see discussion), graciously made available by G.D. d'Mello from the CDE, Porton Down, UK.

Nine9 days later another performance test was carried out 30 min after saline. An injection with the same carbamate at a different dose-level was given again 2 days later. The effects obtained were, in some cases, irregular and difficult to interpret. They were probably influenced by the effects of the first injection with carbamate (state-dependence or tolerance effects). Therefore, the effects following the second injections were omitted from the results. Also, the animals could no longer be considered naive to drugs.

*) Training of the discrimination task was started later than training of the hand-eye coordination and reaction time tasks. In series 1, training of the discrimination task started at session 19 and in series 2 at session 43.

SERIES 2

The tests in session 92 (session 1 in the figures) were control tests; no injection was given. In session 93, the effect of saline was tested again, followed 2 days later by the injection of a carbamate; the same time intervals were used between injection and testing as in series 1. As mentioned above in section II-3, training and testing, hand-eye coordination was tested in the same way as in series 1, but for discrimination performance, the test had been changed. As in series 1, also in series 2 the tests were repeated; 5 days after session 4 in the figures, saline was injected and 2 days later the same carbamate was injected at a different dose-level. Again the effects were erratic and were omitted from the results, for the same reasons mentioned under series 1.

II-5 CHEMICALS

All injections were performed with sterile solutions; the skin was disinfected at the injection site with 70% ethyl alcohol. Pyridostigmine bromide (Mestinon) was obtained from Hoffman La Roche BV, Mijdrecht, The Netherlands. Physostigmine (eserine sulphate) was obtained from Nutritional Biochemical Corporation, Cleveland, Ohio, USA.

II-6 STATISTICS

In view of the relatively small number of animals that could be tested so far, statistical analysis of the results was not meaningful and has been omitted.

III. RESULTS

The layout of figures 1-9 has been kept the same: in each panel of the figures, the effect of a treatment on a specific parameter is plotted for individual animals, in comparison with the mean (± S.E.M.) values of sessions 1-3 and 5-8 for all animals tested. For obvious reasons, averaging the results of session 4 was not meaningful, since in that session the effects of the various treatments were tested. Adding an extra saline-treated control animal at session 4 was carried out to have a simultaneously tested control animal. This was perhaps a bit overdone, since two or three sessions before all, animals had already been tested 30 min after saline, and, even before that, saline injections followed by testing had taken place several times to accustom the animals to the injection routine. As can be seen from the averaged results in each graph, the saline injections caused no changes in the performance of the animals.

Hand-eye coordination

Since the same procedure was used to test hand-eye coordination in series 1 and series 2, these results were combined as shown in figure 1. A dose of pyridostigmine of 0.2 mg/kg i.m. caused a substantial performance decrement in the two animals tested. After a dose of 0.4 mg/kg in one animal, the decrease in performance was not larger but lasted longer.

With physostigmine, performance in one animal after an injection of 0.02 mg/kg i.m. was not affected, whereas the performance of the other animal dropped to zero. Following higher doses of physostigmine (0.04 or 0.08 mg/kg), performance also dropped to zero.

Reaction time

For reasons mentioned in section II, materials and methods, this test in its original form was dropped and replaced by a substitute (motor speed), which was incorporated in the discrimination test used in series 2. For the sake of completeness, the results of the reaction time test in series 1 are shown in figure 2. The results are expressed as the number of times (out of 10) that the animal obtained the reward within 2.00 sec. This rather insensitive way of expressing the results was used because of the variability of the reaction times within those 10 trials. Consequently, as shown in figure 2, the only decrement of performance was seen in an animal that was injected with physostigmine in a dose of 0.08 mg/kg, i.m.

Discrimination performance

For series 1, the results of the discrimination tests are shown in figures 3 and 4. The discrimination performance scores (H/N x 100%) in figure 3 show that the performances of one animal treated with 0.02 mg/kg physostigmine and another with 0.2 mg/kg pyridostigmine were not disturbed. However, when the dose of each carbamate was 4 times higher, i.e., 0.08 mg/kg physostigmine (one animal) and 0.8 mg/kg pyridostigmine (one animal), a clear drop of performance was found (see figure 3). Essentially, the same effects were found for the number of attempts of these animals, as can be seen in figure 4. For series 2, the results of the operant discrimination tests are shown in figures 5-9. The discrimination performance scores [(H/pg + pa) x N/20], as shown in figure 5, were decreased by 0.02 mg/kg physostigmine in one animal and by 0.04 mg/kg of this carbamate in another. Pyridostigmine, at a dose of 0.2 mg/kg in one animal, or at a dose of 0.4 mg/kg in another, was ineffective.

The effects on the other parameters measured follow the same trend, with the exception of one observation which, at this point in the experimentation, is only of limited importance. This is the observation that the animal treated with 0.02 mg/kg physostigmine first chose the correct handle in two successive trials. In the third trial, it first chose the wrong handle, got no access to the reward and stopped trying altogether. As a result of the formula used, the score for correct/total choices was 67% (see figure 6). The choice time for those two correct choices were much longer than 1.00 sec, hence its choice times dropped to zero (see figure 7). Because it was not successful at all in obtaining the reward, and, therefore, the number of hits was zero, its motor speed (time between window open and retrieval of reward), and its discrimination performance (expressed by H/(pg + pa) x N/20), was also zero, as can be seen in figures 8 and 5, respectively. In line with the result obtained with 0.02 mg/kg physostigmine, the animal injected with 0.04 mg/kg stopped performing altogether after having pulled the wrong handle once.

The only animal that had overt symptoms upon close observation was the animal injected with 0.08 mg/kg physostigmine. It showed slight hypersalivation.

IV. DISCUSSION

At this stage of experimentation, our remarks, insofar as they concern the data obtained, should be regarded as speculative or merely suggestive.

Although conclusions have to be postponed until more animals are tested, it would seem that a dose of pyridostigmine as low as 0.2 mg/kg (760 nmol/kg) i.m. is still above the no-effect level. It appeared that this dose caused a clear decrement in the hand-eye coordination of two animals (see figure 1), whereas it did not affect discrimination performance, either in the test used for one animal in series 1 (see figure 3) or in the different discrimination test used for one animal in series 2 (see figure 5). In series 2, a dose of 0.4 mg/kg pyridostigmine also did not cause a decrement of discrimination performance in the one animal injected with this dose. In the case of physostigmine, a dose of 0.02 mg/kg (60 nmol/kg) caused a decrement of hand-eye coordination in one of the two animals tested (see figure 1). In the discrimination test of series 1, this dose had no effect (see figure 3), whereas in the operant discrimination test of series 2, this dose caused a decrease of performance to zero level in the one animal tested (see figure 5). This might indicate, that the no-effect level for physostigmine is also (slightly?) lower than 0.02 mg/kg i.m.

In view of the data of Scott and d'Mello (personal communication) showing that in terms of inhibition of acetylcholinesterase in red blood cells the two carbamates are approximately equipotent, it is interesting to note that so far it does not seem unlikely that the dose of pyridostigmine needed to cause a deficit in hand-eye coordination will be higher than the dose of physostigmine needed to obtain a similar effect (see figure 1). A much larger difference seems to exist between the doses of both carbamates needed to cause a decrement in discrimination performance. Perhaps this is due to the fact that during hand-eye coordination (with a moving target), a greater demand is made on the fine-regulation of muscle-tonus, whereas during discrimination performance (stationary target) a greater demand is made on CNS processes (discrimination, choice-process, decision). Since pyridostigmine, due to its quaternary nitrogen, is believed to hardly penetrate the brain, this might explain why discrimination performance seems even less susceptible to pyridostigmine than does hand-eye coordination.

Although a greater variability was expected with marmosets of different sources than with rats of a pure strain bred in our laboratory, after two series of experiments it was felt that the numbers of animals of a group that ultimately reached an acceptable performance level for testing drug effects was too small. Therefore, we invited E.A.M. Scott and G.D. d'Mello to come to our laboratory with the aim of letting these experienced investigators make a critical analysis of the data obtained and especially of the behavioral training procedures used so far. Both investigators have at least a decade of experience with marmosets and their behavior.

These analyses and the subsequent discussions during their 2-day visit led to some changes in the training and testing procedures. Following the discussions with these colleagues from Chemical Defence Establishment (CDE), Porton, UK (see discussion), for the next series it was decided:
1) To split the tasks: the animals were tested either for hand-eye coordination or for discrimination performance;
2) To increase the number of trials per day, 40 instead of 20 trials for the discrimination task and 40 instead of 10 for the hand-eye coordination test;

3) To introduce one stationary trial at the beginning of each hand-eye coordination test; and
4) To introduce a cutoff time of 30 sec for each presentation of the handle; thereafter the handle is withdrawn, and a new trial is started as mentioned before. Their main advice was to split the tasks to avoid confusing the animals and to train the animals more rigorously by increasing the number of trials from 10 to 40 per day for series 3. In their visually guided reaching test, they used 80 trials per day. The number of trials for the discrimination test was also increased from 20 to 40. Most likely this will lead to more rapid acquisition and a faster progress of experimentation.

Pending the determinations in our own laboratory of the AChE-activities following administration of these carbamates, we made use of data graciously made available by our British colleagues. In their experiments with marmosets, they found that 15 min after i.m. administration, a dose of 0.025 mg/kg pyridostigmine caused an average inhibition of red blood cell AChE-activity of approximately 30%, whereas a dose of 0.05 mg/kg caused an average inhibition of approximately 45% and 0.1 mg/kg, an average inhibition of approximately 50%. Similarly, after i.m. doses of 0.02, 0.04 or 0.08 mg/kg physostigmine, they found average inhibitions of approximately 25%, 40% or 60%, respectively. This means that these two carbamates are approximately equipotent when it comes to inhibition of peripheral AChE (RBC-AChE), both on a mg/kg and a μmol/kg basis.
However, the doses at which they find behavioral effects differ: pyridostigmine has to be given at approximately a 10 times higher dose than physostigmine to obtain a significant deficit of performance in their visually guided reaching task.

The present tasks, used in series 1 and 2, may be more difficult than those used by Scott and d'Mello and therefore may be more sensitive to the disruptive effects of carbamates. In fact, our British colleagues did not find significant deficits in a fairly large number of marmosets using their conveyer belt task (visually guided reaching task) following i.m. injections of 0.2 mg/kg pyridostigmine or 0.02 mg/kg physostigmine (personal communication, G.D. d'Mello and E.A.M Scott). When they tested increasing doses, pyridostigmine started to cause a significant performance deficit at a dose of 0.8 mg/kg and physostigmine at a dose of 0.08 mg/kg. These doses are approximately 4 times higher than those that caused deficits in the few animals tested so far in the present experiments. The finding that a much larger dose of pyridostigmine than of physostigmine had to be given to cause significant behavioral deficits (as found by our British colleagues) seems also evident in the data of the present experiments, although more animals need to be tested.

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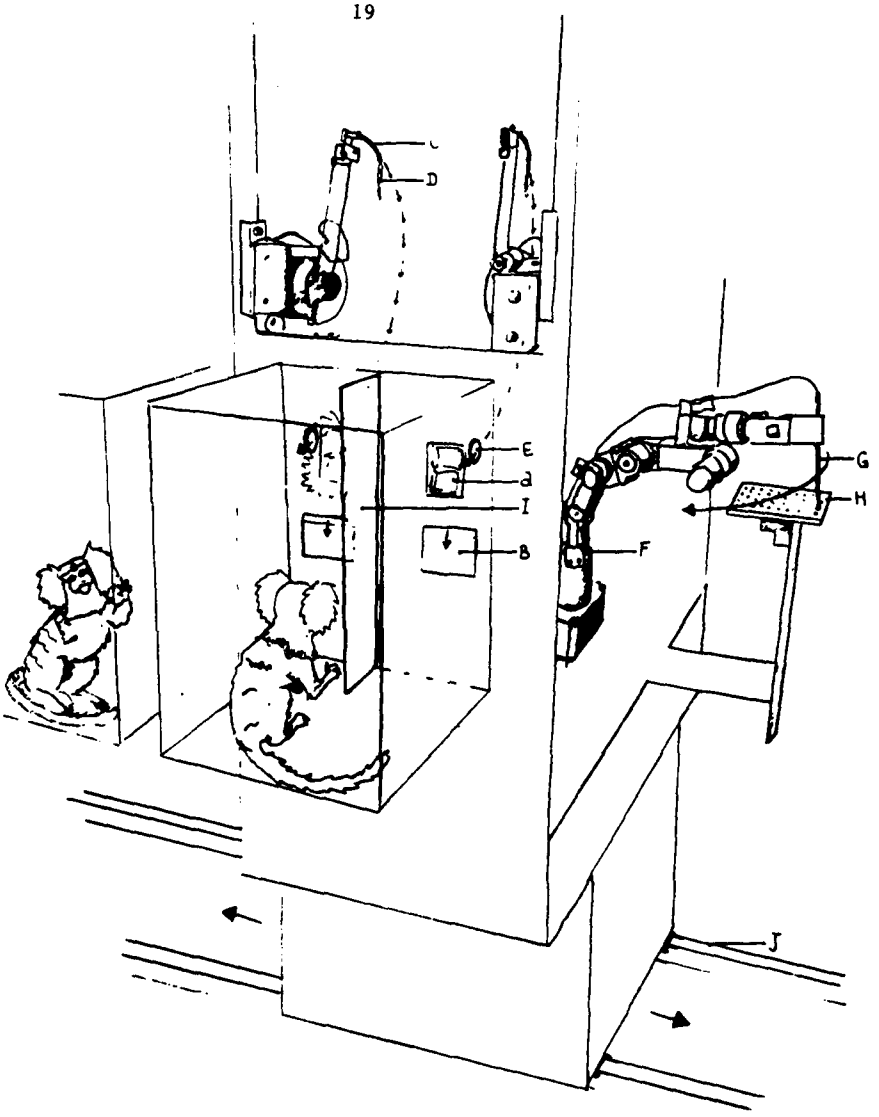

FIGURE 0.
A drawing of the experimental setup. A) the alpha-numerical display, B) the sliding door in the window, C) the motor-driven handle with the chain, (D) attachment which moves into the cage through the hole marked E in the test-panel, F) the robot-arm, G) the suction tube that picks up a reward from one of the 120 wells in the plateau marked H, I) the partitioning screen projecting into the cage, preventing the animal from sitting in the middle between the windows, and J) the rails on which the platform with the robot moves from one cage to another. Some essential pieces of equipment, such as the two TV-cameras, had to be left out to keep the illustration as clear as possible.

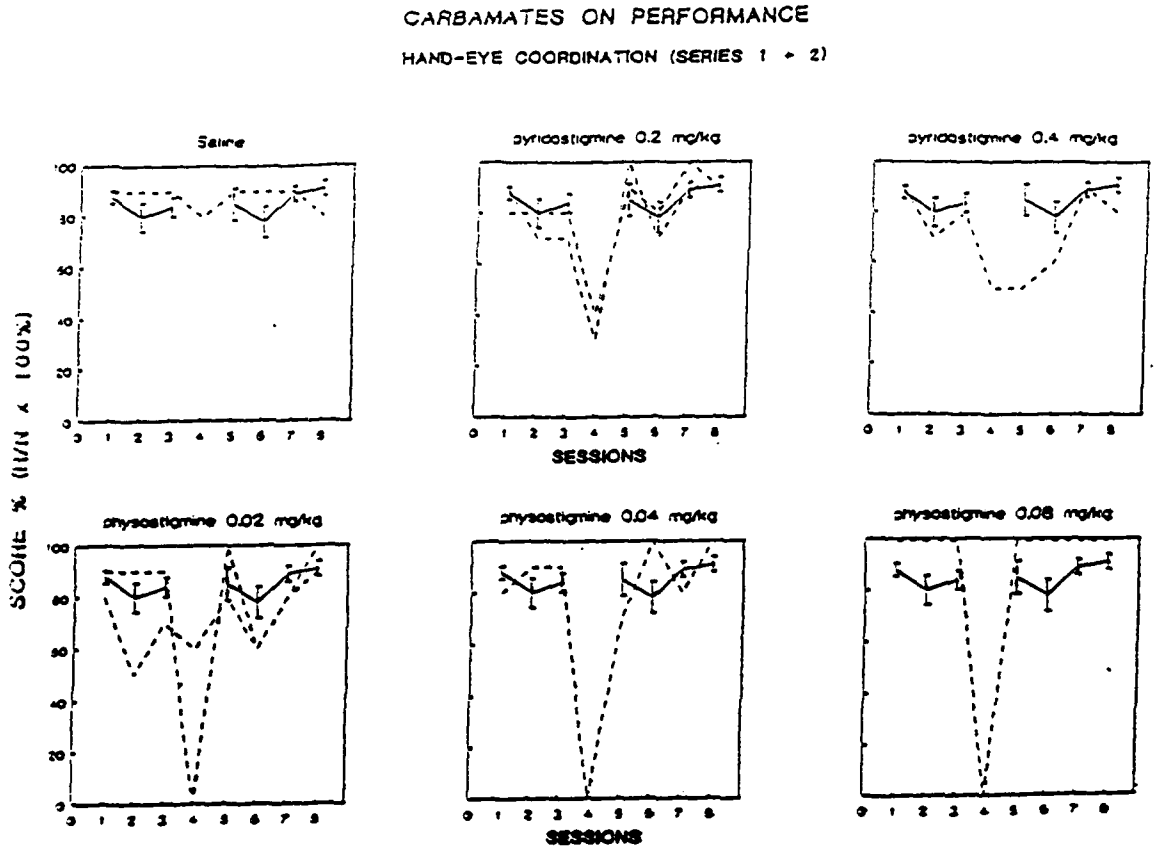

FIGURE 1.
The acute effects of saline, pyridostigmine and physostigmine on the performance of marmosets in an acquired robot-tested hand-eye coordination task. In each graph, the individual data are plotted against the mean (± S.E.M.) performance in sessions 1-3 and 5-8 of all animals tested (in session 4) in series 1 and 2. H = the number of "hits" and N = the number of trials completed (see section II-3). Behavioral testing started 30 min after the injection of pyridostigmine and 20 min after the injection of physostigmine. In session 1 or 2 all animals received an i.m. injection of saline. The doses of 0.2 mg/kg i.m. pyridostigmine and 0.02 mg/kg physostigmine are above the no-effect level.

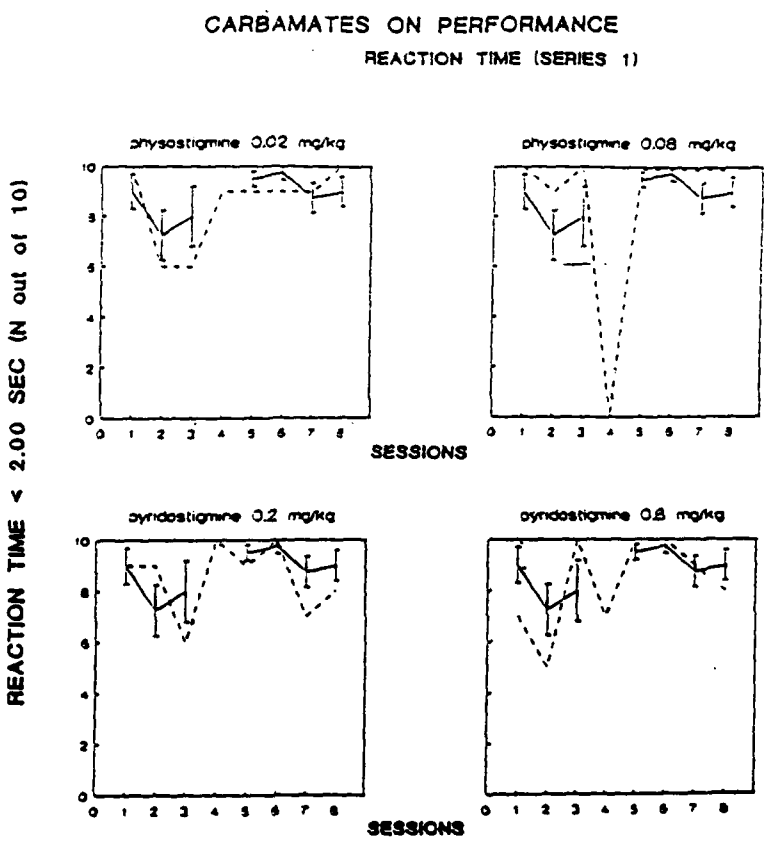

FIGURE 2.
The effects of pyridostigmine and physostigmine on the performance of the reaction time task in series 1. The data are presented in the same manner as in figure 1. Only a relatively high dose of physostigmine (0.08 mg/kg i.m.) caused a deficit of performance. This task was dropped and replaced by a substitute task, motor speed in series 2, see figure 8.

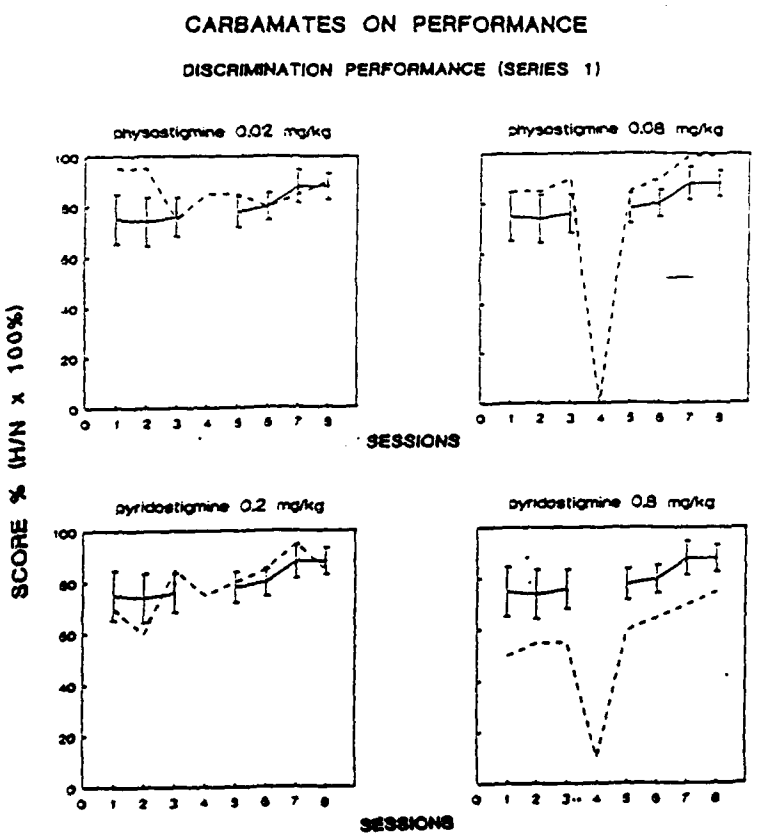

FIGURE 3.
The effects of pyridostigmine and physostigmine on the performance of a discrete-trial two-choice visual discrimination task. The data, for series 1 only, are presented in the same manner as in figure 1. Only the relative high doses of 0.8 mg/kg i.m. pyridostigmine and 0.08 mg/kg i.m. physostigmine caused deficits of performance. This task was transformed into an operant task in series 2.

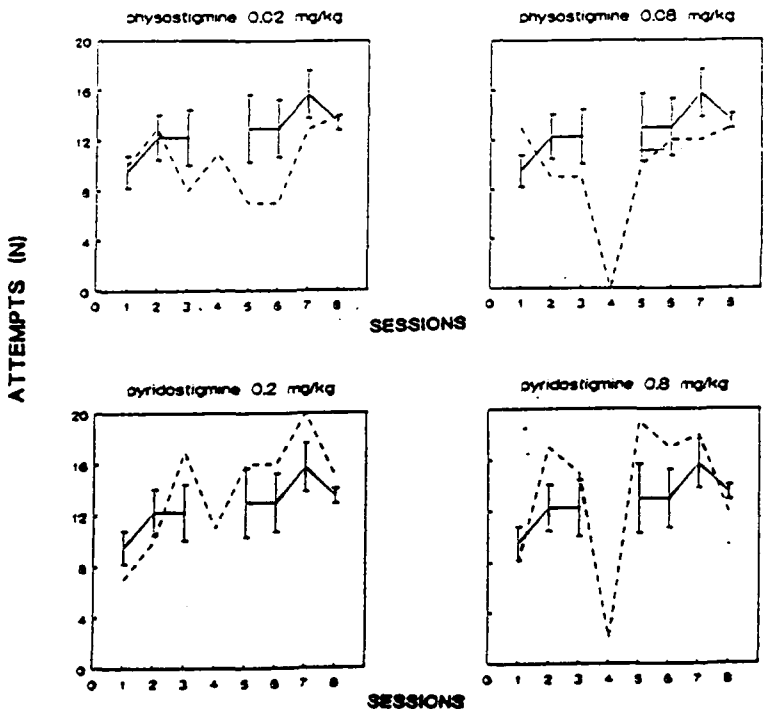

FIGURE 4.
The effects of pyridostigmine and physostigmine on the number of attempts during discrimination behavior in session 1, shown in figure 3. Data presentation, for series 1 only, is the same as in figure 1. The effects are comparable to those seen in figure 3.

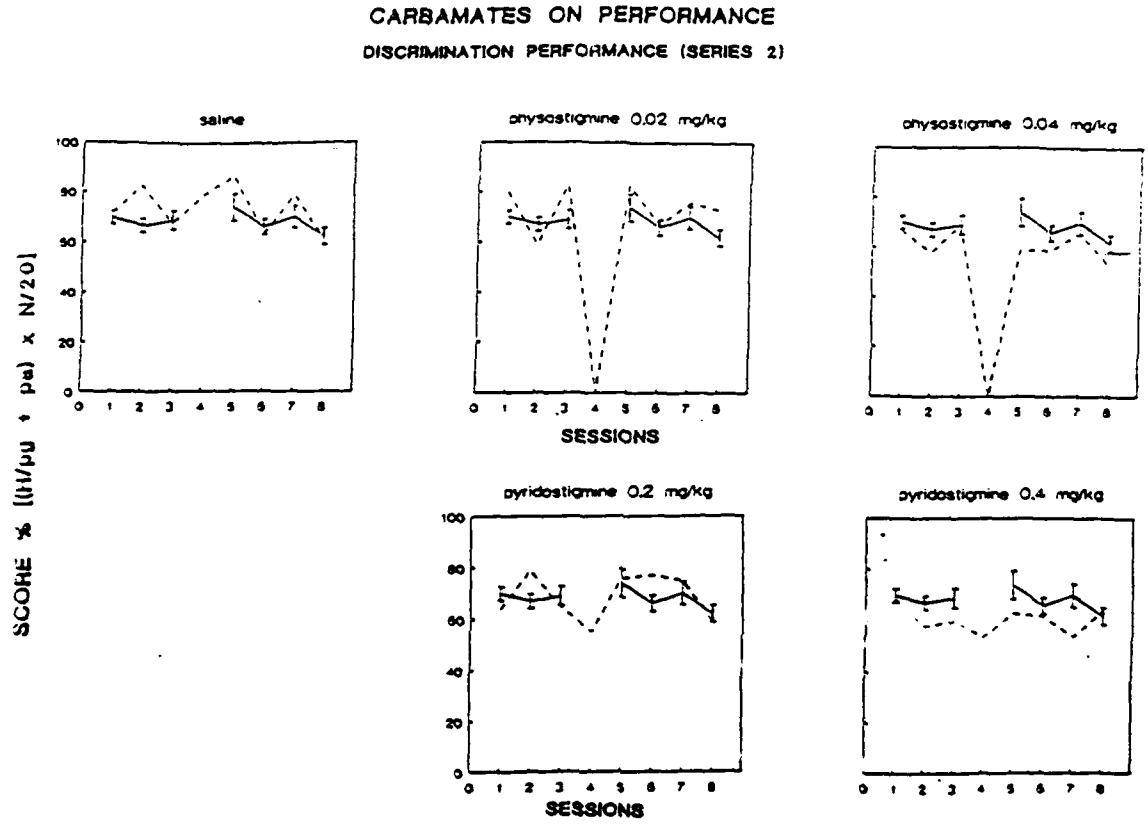

FIGURE 5.
The effects of saline, physostigmine and pyridostigmine on performance of the operant discrete-trial two-choice visual discrimination task. The data, for series 2 only, are presented in the same manner as in figure 1. For definitions of the number of hits (H), the number of trials completed (N), the number of correct first choices (Pg) and the number of wrong first choices (Pa) see section II-3. In contrast to the task in series 1 (see figure 3), the marmosets had to pull a (correct) handle to gain access to the reward. Physostigmine caused a profound deficit in performance at doses of 0.02 - 0.04 mg/kg, pyridostigmine had no effect in doses of 0.2 - 0.4 mg/kg. As in earlier sessions, the injection with saline in session 4 had no effect.

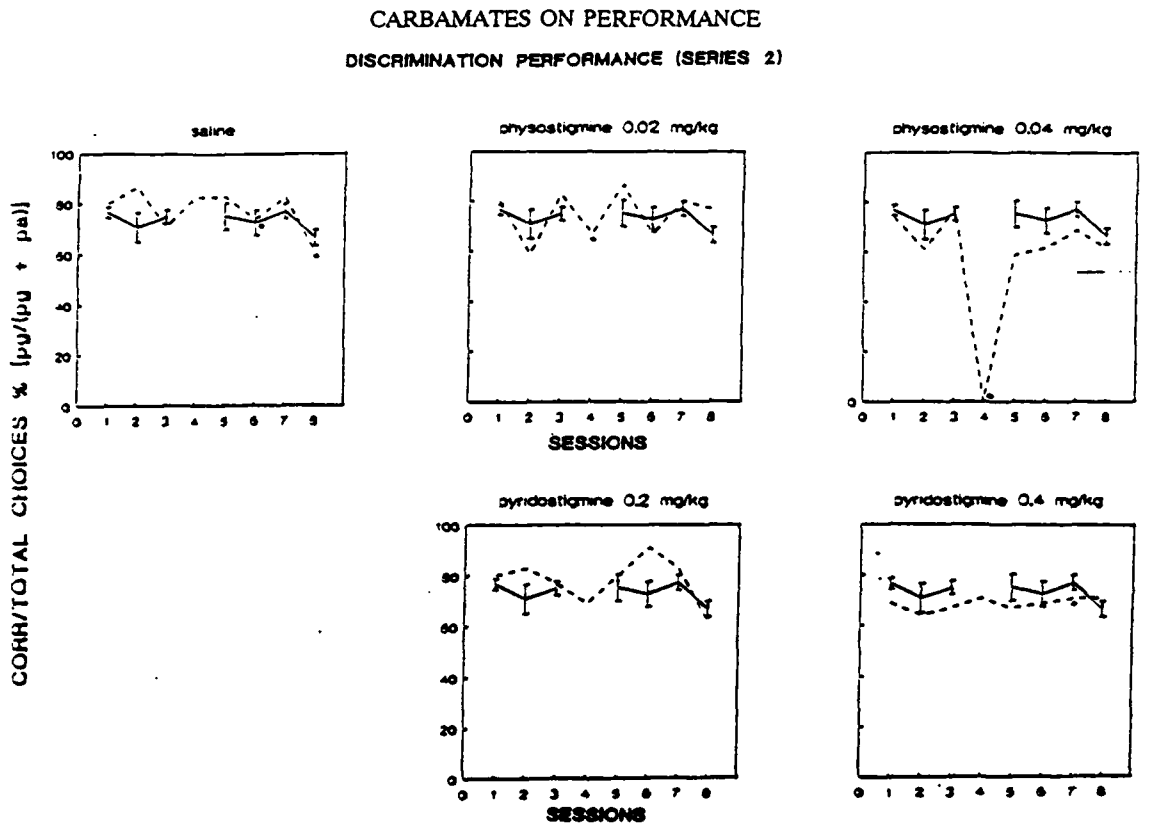

FIGURE 6.
The effects of saline, physostigmine and pyridostigmine on the ratio of the number of times the animal first chose the correct handle (Pg) to the total number of choices (Pg + Pa) made. The lowest dose of physostigmine tested had no effect; pyridostigmine had no effect in the doses tested. The asterisks indicate that in that session the animals did not complete all their trials.

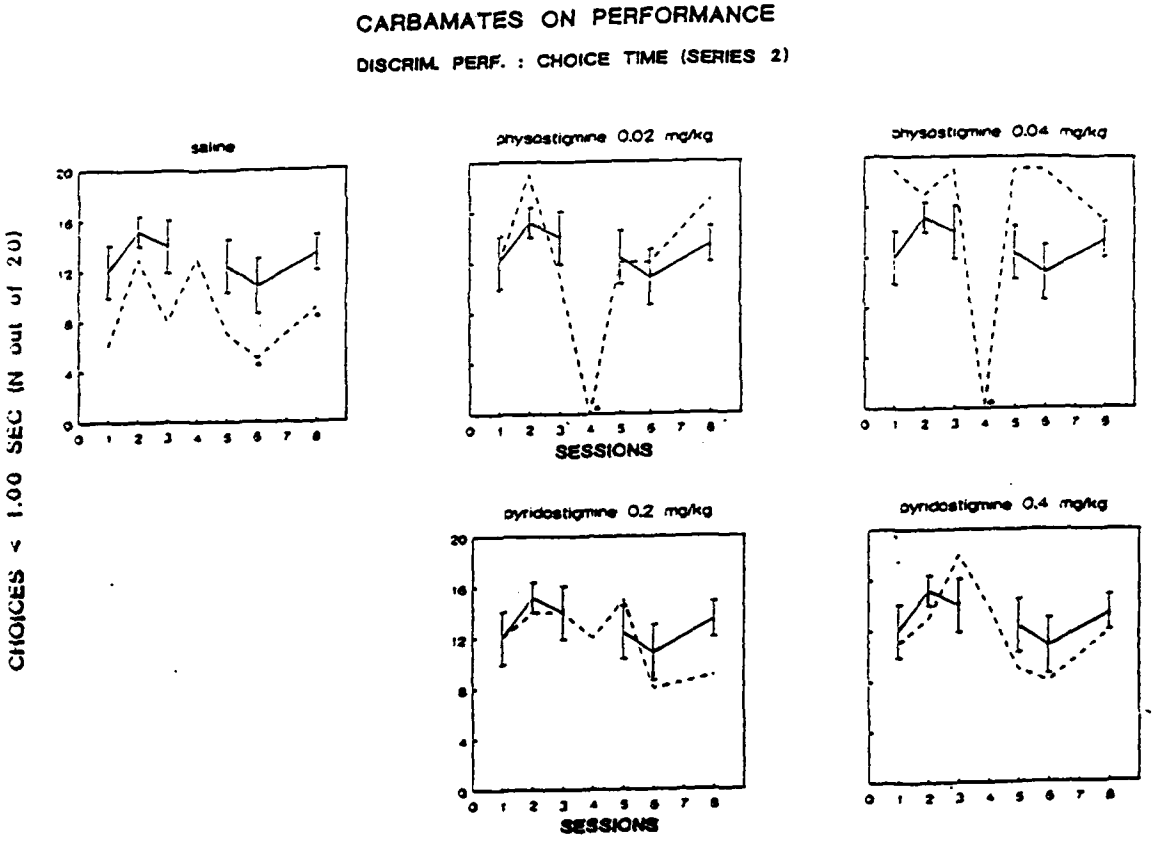

FIGURE 7. The effects of saline, physostigmine and pyridostigmine on the time it took the animal during its first choice of the correct handle (decision time for the correct choices). Essentially the same effects were seen as in figure 5. The asterisks indicate that the animals did not complete all their trials.

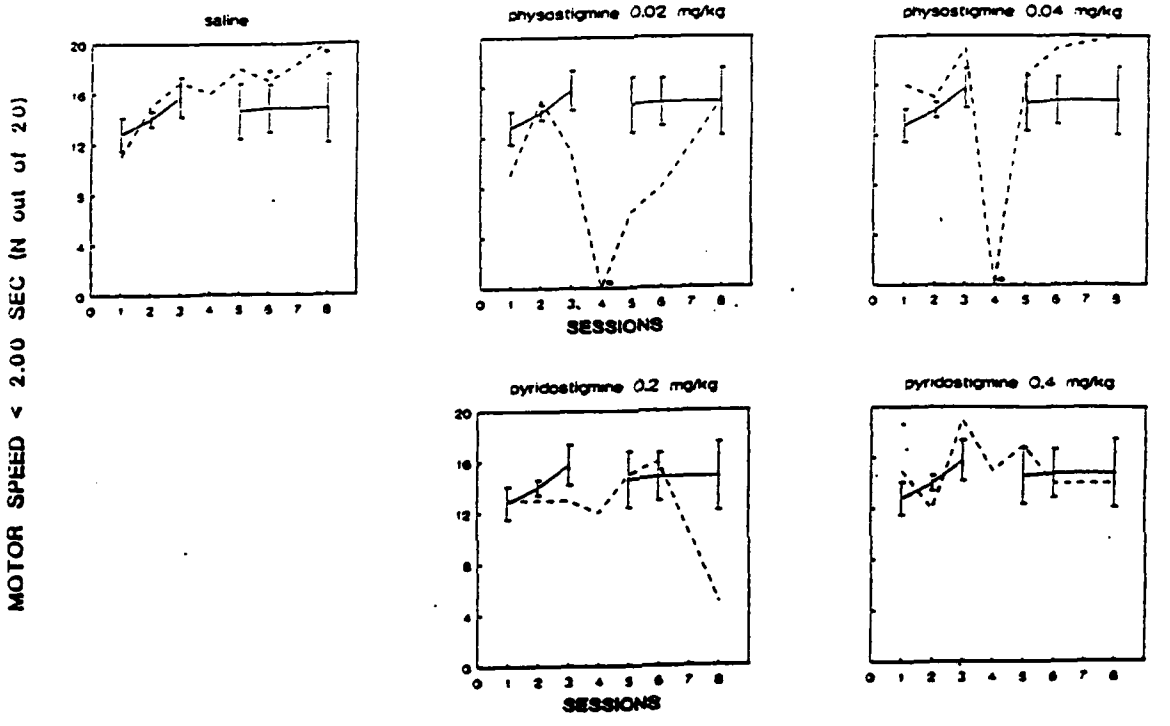

FIGURE 8.
The effects of saline, physostigmine and pyridostigmine on the time it took the animal to retrieve the reward from the moment it became accessible, which represents the speed with which the animal reacts, or motor speed. Compare with figures 5 and 7. The late decrease in motor speed after 0.2 mg/kg pyridostigmine is not understood.

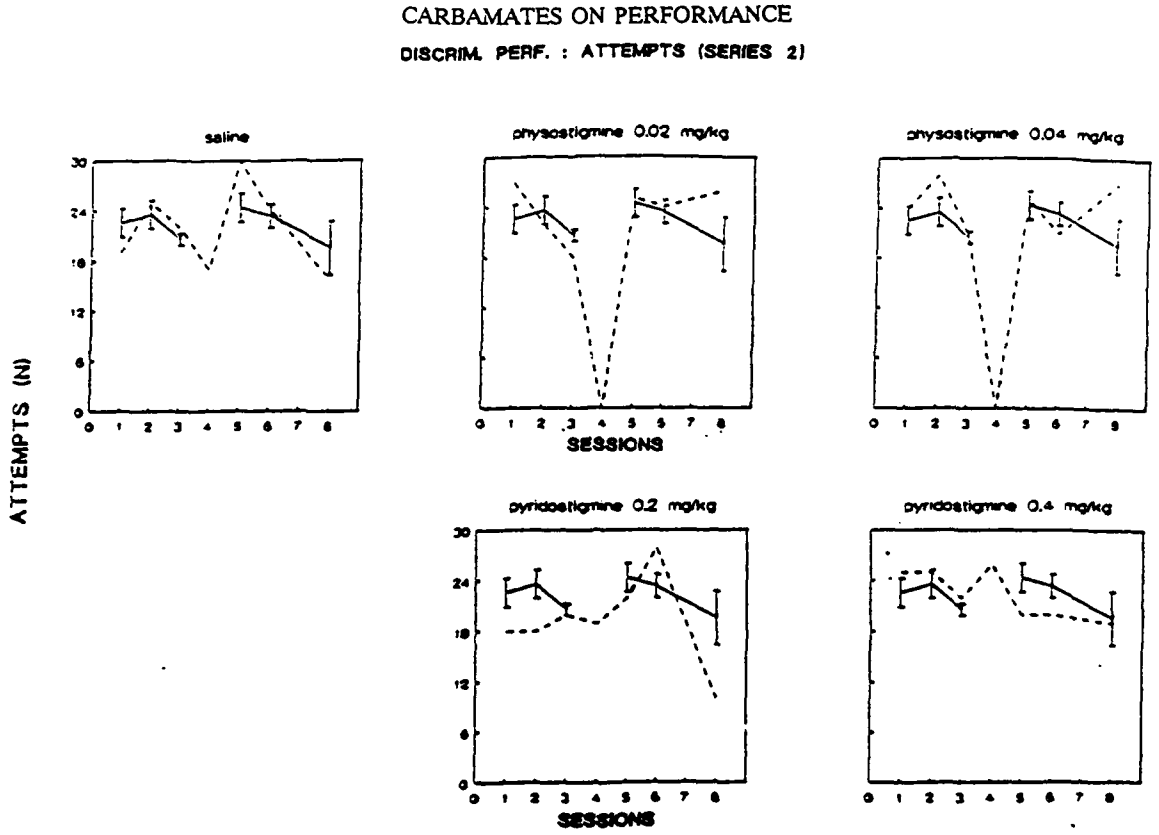

FIGURE 9.
The effects of saline, physostigmine and pyridostigmine on the number of attempts. Essentially the same results were obtained with these doses of both carbamates as in figure 5.